[5] J.M. Santini, I.S. Lindsay, R.D. Schnagl, J.M. Macy, Appl. Environ. Microbiol. 66 (2000) 92–97.

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Exploring the coupling between electron transfer and protein dynamics in photosynthetic reaction centers embedded into dehydrated amorphous matrices

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The interplay between protein dynamics and electron transfer (ET) has been extensively investigated in the bacterial photosynthetic reaction center (RC) from *Rhodobacter sphaeroides* by hampering RC internal motions at low temperatures [1]. Alternatively, the RC dynamics can be inhibited at room temperature by incorporating the RC into dehydrated trehalose matrices [2]. In the glasses the recombination kinetics of the charge separated state $P^+Q_A^-$ are accelerated and distributed in rate as compared to solution, mimicking at room temperature the effects observed at 10 K in water-glycerol. This is taken to indicate inhibition of the RC relaxation from the dark- to the light-adapted conformation, as well as of the RC thermal fluctuations [1,2]. We proposed that the inhibition is mediated by residual water molecules of the RC hydration shell which bridge protein surface groups with trehalose molecules of the matrix by forming a network of multiple hydrogen bonds [3]. Consistently, similar effects have been observed also in RC films dehydrated in the absence of sugar [4]. However, striking differences are found between RC-trehalose glasses and RC-films: (a) The thermal stability of the RC is tremendously enhanced in the trehalose matrix. (b) In RC-trehalose matrices, the $P^+Q_A^-$ recombination after a few seconds of continuous photoexcitation is only partially decelerated as compared to the one recorded after a laser flash, whereas in dried RC films a comparable period of continuous illumination leads to a total recovery of the kinetics observed in the hydrated system. These data indicate that in films the protein dynamics can be easily regained, in contrast to trehalose glasses, which reveal much stronger structural constraints. We are extending these studies to a series of RC mutants characterized by a widely altered P^+/P midpoint potential relative to wild-type [5]. In these mutants, the acceleration of $P^+Q_A^-$ recombination induced by cooling to 10 K in the dark decreased with increasing midpoint potential [6]. Interestingly, incorporation into dehydrated trehalose matrices causes instead acceleration of the kinetics by the same factor for all P^+/P midpoint potential values.

References

[1] McMahon, et al., Biophys. J. 74 (1998) 2567.
[2] Palazzo, et al., Biophys. J. 82 (2002) 558.
[3] Francia, et al., JACS 130 (2008) 10240.
[4] Malferrari, et al., J. Phys. Chem. B 115 (2011) 14732.
[5] Lin, et al., PNAS 91 (1994) 10265.
[6] Ortega, et al., Biochemistry 35 (1996) 3354.

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Ruthenium Photoinitiation of Electron Transfer in the *Rb. Sphaeroides* Cytochrome bc_1 Complex: Rotational dynamics of the iron-sulfur protein, diffusion of quinone between the Q_o and Q_i sites, and linkage between the two monomers

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Electron transfer within WT *R. sphaeroides* cytochrome bc_1 was studied using a ruthenium dimer, Ru_2D, to rapidly photo-oxidize heme c_1 within 1 μs. A range of different conditions including viscosity, temperature, inhibitors and substrates was used to study electron transfer between the iron-sulfur protein (ISP) and heme c_1, as well as electron transfer through the *b* hemes following turnover. Electron transfer between the ISP and heme c_1 was biphasic, with an initial fast rate, k_1, of 80,000 s^{-1}, and slow rate, k_2, of 3,500 s^{-1}. k_1 was found to decrease significantly with increasing viscosity, suggesting that k_1 is gated by rotational diffusion. k_1 and k_2 were fully inhibited by half an equivalent of the stigmatellin, indicating that both ISP were unable to rotate toward the *c*-state when stigmatellin is bound to only one Q_o site of the monomer. Electron transfer through the *b* hemes following Q_o site turnover was observed with an initial reductive phase with rate constant, k_2, of 3,500 s^{-1}, followed by a slower re-oxidation of heme b_H with a rate constant, k_4, of 500 s^{-1}. When both hemes b_H were pre-reduced, two equivalents of heme b_H were re-oxidized per quinol oxidized at the Q_o site. This suggests that the quinone product of Q_o site turnover diffuses through the hydrophobic interior of the complex to act as substrate at the Q_i site, and that electrons are transferred across the hemes b_L with a rate constant of at least 500 s^{-1}. It is also shown that k_4 is not affected by antimycin until at least half an equivalent of the inhibitor is present. Supported by NIH grants GM20488 and 8P30GM103450.

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Sub-micron scale distribution of electron transport compelxes in bacterial membranes, and its influence on electron transfer pathways

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We use fluorescence microscopy in combination with GFP-tagging to investigate the distribution and dynamics of electron transport complexes in bacterial bioenergetic membranes *in vivo* [1-4]. Membranes investigated include the cytoplasmic membrane of *Escherichia coli* [1], the cytoplasmic membrane of the atypical cyanobacterium *Gloeobacter violaceus* [2] and the thylakoid membranes of the cyanobacteria *Synechococcus* sp. PCC 7942 [3] and *Synechocystis* sp. PCC 6803 [4]. Electron transport complexes visualised include the photosynthetic reaction centres [2-4], respiratory complexes [1-3]